GENETIC POLYMORPHISMS AND BREAST CANCER RISK: A REVIEW

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Recent studies have provided important new insights into the molecular epidemiology and genetics of breast cancer (1-8). Much of this research has focused on the aggregation of breast (and ovarian) cancer within high-risk families, as well as on the role of major cancer susceptibility genes, such as *BRCA1*, *BRCA2*, and *p53* germline mutations (8-10). Putative associations, such as that between heterozygosity for *ATM* (a gene mutated in ataxia telangiectasia) and the risk of breast cancer, have also been studied (11-16). These susceptibility genes account for less than 5% to 10% of cases of breast cancer in the population, however. Other genetic factors, when one considers their interactions with environmental risk factors for breast cancer, may have greater public health importance (9).

The search for other genetic markers for susceptibility to breast cancer has led to an increasing number of epidemiologic studies of relatively common genetic polymorphisms that may have a role in the metabolism of estrogens or in the activation or detoxification of drugs and environmental carcinogens (17-20). The existence of low-penetrance genetic polymorphisms may account for why some women are more sensitive than others to environmental carcinogens such as replacement estrogens. For example, genetic polymorphisms for cytochrome P450 enzymes and N-acetyltransferase 2 have been examined in relation to cigarette smoking and breast cancer susceptibility in women (17, 19). *CYP17*, a gene that codes for a cytochrome P450 enzyme involved in the metabolism of estrogen, has been associated with young age at first menstruation and increased risk of breast cancer (20). The number of cases of breast cancer that are

attributable to such genetic polymorphisms (in combination with environmental exposures) is likely to be much higher than the number of hereditary cases caused by mutations of high-penetrance genes such as *BRCA1* and *p53* (9). The genetic polymorphisms that may be linked to breast cancer are much more common in the population than are the high-penetrance cancer susceptibility genes (9).

In this paper, we review research on genetic polymorphisms that may have an etiologic role in breast cancer. Included in this review are associations identified in molecular epidemiology studies, the consistency of findings reported to date, and interactions with environmental factors. Suggestions for further research are also offered.

CYTOCHROME P450 ENZYMES

Cytochrome P450 enzymes make up a multiple-gene "superfamily" that plays an important role in steroidogenesis and in the activation or detoxification of environmental chemicals such as polycyclic aromatic hydrocarbons, benzo(a)pyrene, arylamines, and heterocyclic amines (17, 21). Although P450 cytrochromes can provide a line of defense against exposure to environmental chemicals, carcinogens are more commonly activated by P450 metabolism. Cytochrome P450 enzymes are expressed primarily in the liver and other tissues (17, 21).

Molecular epidemiology studies of breast cancer (as well as cancer of the lung, bladder, colon, and other sites) have examined associations with P450 cytochrome genotypes, such as *CYP1A1*, *CYP2D6*, and *CYP17* (17, 18). Earlier studies, carried out before the availability of DNA tests for *CYP1A1* and *CYP2D6*, examined the activity of the

corresponding polymorphically expressed enzyme. Many other cytochrome P450 enzymes (including those coded for by *CYP1A2*, *CYP1B1*, *CYP2A*, *CYP2B*, *CYP2C*, *CYP2E1*, *CYP3A*, and *CYP4B1*) are involved in the activation or detoxification of drugs and other xenobiotic compounds (17).

CYP1A1 (aryl hydrocarbon hydroxylase gene)

The *CYP1A1* gene is located on chromosome 15q and codes for Aryl hydrocarbon hydroxylase (AHH)(17).   AHH metabolizes polycyclic aromatic hydrocarbons and has been found in breast tumor tissue (22). AHH is strongly inducible; that is, it exhibits greater enzymatic activity with increasing exposure to substrates (17). AHH catalyzes the monooxygenation of polycyclic aromatic hydrocarbons to phenolic products and epoxides that may be carcinogenic (17, 23). The enzyme is also involved in the conversion of estrogen to hydroxylated conjugated estrogens, such as 2-hydroxyestradiol (24). Because of the reported link between estrogens and the risk of breast cancer, the role of AHH in both carcinogen activation and estrogen metabolism supports the biologic plausibility of associations between genetic polymorphisms of the *CYP1A1* gene and the risk of breast cancer (23).

Four polymorphisms of the *CYP1A1* gene that codes for AHH have been identified so far including a MspI restriction fragment length polymorphism (RFLP) of the 3' end of the gene (MspI), an adenine-to-guanine mutation in exon 7 of this gene, which causes an isoleucine-to-valine substitution (Ile-Val), and a polymorphism of the *CYP1A1* gene identified among African Americans (AA) which results from a single A-T to G-C transition

in the 3' noncoding region (23, 25, 26). The frequencies of the MspI and Ile-Val

polymorphisms vary considerably by race; frequencies are higher among Japanese and

Hawaiian populations than among Caucasians and African Americans (27).

CYP1A1 has been associated with cancer of the lung, colon, breast, and other

sites (18). Molecular epidemiology studies of CYP1A1 and breast cancer are

summarized in Table 1.   All five of these studies examined the Ile-Val polymorphism (23,

25, 26, 28, 29). Taioli et al. (25) and Ishibe et al. (26) also looked at the MspI

polymorphism. Not shown in Table 1 is that Taioli et al. (25) also examined AA

polymorphism among African Americans in New York City, but here they found no

association with breast cancer risk (n=21 cases and 86 controls, odds ratio [OR] = 1.2,

95% confidence interval [CI] = 0.3-5.3). In studies carried out to date, the point estimates

of the odds ratios for the association of CYP1A1 polymorphisms and breast cancer have

mostly been close to one (Table 1).

Using a nested case-control design, Ishibe et al. (26) examined associations

between breast cancer and polymorphisms in the CYP1A1 gene. The study involved 466

women with incident breast cancer and 466 age-matched controls identified in the Nurses'

Health Study. The overall results from this study provided no evidence of a positive

association between either the MspI or the Ile-Val polymorphism and the risk of breast

cancer (Table 1). However, the risk of breast cancer was higher among women who had

begun to smoke before the age of 18 and carried the CYP1A1 MspI variant genotype

(n=14 cases and 3 controls) than among nonsmokers who carried the homozygous wild-

type allele for this polymorphism (n=162 cases and 176 controls, OR = 5.6, 95% CI = 1.5-21.3)(26). These results suggest that cigarette smoking early in life may increase the risk of breast cancer in a subpopulation of genetically susceptible women.

Ambrosone et al. (28) found a positive but not significant association between breast cancer and the Ile-Val polymorphism in a population-based, case-control study of breast cancer among postmenopausal women in western New York state (OR = 2.9, 95% CI = 0.5-16.6). The risk of breast cancer associated with the *CYP1A1* polymorphism was highest for those women who smoked up to 29 pack-years (n=31 cases and 53 controls, OR = 5.2, 95% CI = 1.2-23.6)(28).

CYP2D6 (debrisoquine hydroxylase gene)

The *CYP2D6* gene is located on chromosome 22q and codes for debrisoquine hydroxylase (17, 21), which metabolizes a variety of drugs and other xenobiotics. Like other polymorphically expressed P450 enzymes, the *CYP2D6* gene may activate procarcinogens or, conversely, detoxify carcinogens (17). A number of alleles have been characterized at the *CYP2D6* locus. The " poor-metabolizer" phenotype (*CYP2D6* mutant/mutant genotype), which is rare in Asians, occurs in about 5% to 10% of Caucasians and 2% of African Americans (21).

Molecular epidemiology studies of the *CYP2D6* genotype and its association with breast cancer risk are summarized in Table 2. Based on genotype assays in patients with breast cancer and in controls, both Smith et al. (30) in Great Britain (an update of a study by Wolf et al. (31)) and Buchert et al. (32) in the United States reported no associations.

Buchert et al. (32) found odds ratios of developing breast cancer to be 1.4 (95% CI = 0.8-2.4) for *CYP2D6-WT*/mutant genotype and 1.3 (95% CI = 0.5-3.5) for the *CYP2D6-*mutant/mutant genotype, compared to the *WT/WT* genotype (Table 2). The study was limited by the use of volunteer controls and by the ascertainment of breast cancer cases from a single medical center; all of the patients with breast cancer were Caucasian women. Ladona et al. (33) in Spain found that women carrying the *CYP2D6* mutant allele had a higher risk of breast cancer (OR = 1.7, 95% CI = 1.1-3.1). A limitation of this study was the use of prevalent rather than incident cases of breast cancer.

In contrast to the mostly negative results from studies of *CYP2D6* genotypes, Ladero et al. (34), in a study of debrisoquine hydroxylase phenotypes, found that Spanish women who were poor metabolizers had about a twofold increased risk of breast cancer (data not shown). A study by Pontin et al. (35) in Great Britain also provided some evidence of an association between the poor-metabolizer phenotype and the risk of breast cancer. However, a study by Huober et al. (36) in Germany failed to find such an association.

These inconsistent results may be explained by the limitations of the phenotype assay, by ethnic differences in the frequency of the *CYP2D6* genotype, by uncontrolled confounding factors in some studies, or by population differences in environmental risk factors for breast cancer. Inconsistencies across studies may also be accounted for by small numbers of cases and controls; confidence intervals have often been overlapping.

CYP17 and the metabolism and transport of estrogens

The *CYP17* gene, which codes for a cytochrome P450 enzyme that is involved in the metabolism and transport of estrogen, may also influence the risk of breast cancer in women. In a study of 83 young nulliparous women, Feigelson et al. (37) showed that the *CYP17* genotype is associated with serum estrogen levels. In their study, serum estradiol levels measured on about day 11 of the menstrual cycle were 11% higher among women with the *A1/A2* genotype and 57% higher among those with the *A2/A2* genotype than among those with the *A1/A1* genotype (p = .04) (37). On about cycle day 22, serum estradiol levels were 7% higher among *A1/A2* women and 28% higher among *A2/A2* women (p = .06), and progesterone levels were 24% higher among *A1/A2* women and 30% higher among *A2/A2* women (p = .04) than among *A1/A1* women. The results of this study provide evidence that serum hormone levels are under genetic control.

The *CYP17* gene codes for the cytochrome P450C17α enzyme, which mediates both 17α-hydroxylase and 17,20-lyase activities and functions at key branch points in steroidogeneis (20, 38). Cholesterol may be converted to progestins, androgens, and estrogens by several pathways, the choice of which is determined by the cytochrome P450C17α enzyme (20).

In a nested case-control study of Asian, African American, and Latina women in Los Angeles and Hawaii, involving 174 cases and 285 population controls, *CYP17* genotypes (*A2/A2* and *A2/A1* alleles) were associated with young age at first menstruation and an increased risk of breast cancer (20). The odds ratio associated with the *A2* allele was 2.5 (95% CI = 1.07-5.94) for regional or metastatic breast cancer (n=40 cases). Because the

allele associated with high estradiol levels is common (roughly 40% of the population has it), the risk for breast cancer that is attributable to this genetic polymorphism may be substantial (perhaps as high as 29%, although these preliminary findings have not been confirmed by other investigators (39, 42)).

Helzlsouer et al. (39) found no association between *CYP17* genotype and risk of breast cancer in a nested case-control study of 115 incident cases of breast cancer and 115 controls in Washington County, Maryland. Similarly, Weston et al. (40) found no association between *CYP17* genotype and risk of breast cancer in a hospital-based case-control study 363 women with breast cancer and 240 patient controls in New York City. These authors also found no evidence that *CYP17* genotype is associated with age at menarche. Negative findings were also obtained by Dunning et al. (41) in a case-control study in East Anglia, England involving 835 breast cancer cases and 591 controls (OR = 1.1, 95% CI = 0.9-1.4).

More recently, Haimman et al. (42) prospectively assessed the association between *CYP17* genotype and breast cancer risk in a case-control study nested with the Nurses' Health Study cohort. A total of 463 cases and 618 controls were included in the study. Women with the *A2* allele were not at an increased risk of incident breast cancer (OR = 0.9, 95% CI = 0.7-1.1). The protective effect of later age at menarche was only observed among women without the *A2* allele, however, suggesting a possible interactive effect with *CYP17* genotype.

Possible explanations for these inconsistent findings across studies include

differences in underlying racial distributions and differences in breast cancer stage at diagnosis.

N-ACETYLTRANSFERASE 1 AND 2

The N-acetyltransferase-1 (*NAT1*) and N-acetyltransferase-2 (*NAT2*) genes are located on chromosome 8q (17). Both are polymorphically expressed in a variety of tissues. *NAT2* detoxifies or, conversely, activates aromatic amines such as 4-aminobiphenyl, which is found in tobacco smoke (43). Both phenotypic and genotypic assays for *NAT2* can be used to classify individuals as rapid or slow acetylators. Genetic variants of the *NAT2* gene have been cloned, and several alleles at this locus have been identified; the *F1* allele confers the fast-acetylator phenotype (17, 18). Several variants of the *NAT1* gene have also been identified. The distribution of *NAT1* and *NAT2* alleles differs widely between racial and ethnic groups. Although *NAT2* may be less active than *NAT1* in breast tissue, detoxification of aromatic amines in the liver may also have a role in protecting women against breast cancer (17).

Studies of the *NAT2* phenotype and breast cancer susceptibility have produced inconsistent results (44-46). More recent studies that have examined *NAT2* genotypes in breast cancer, on which we focus here, have also had inconsistent findings (43, 47-49). Moreover, the inconsistent findings among studies of *NAT2* and breast cancer have often involved associations occurring in opposite directions, for example, for slow alleles versus fast alleles or for postmenopausal women versus premenopausal women.

Agundez et al. (47) examined the association between N-acetyltransferase polymorphisms and the risk of breast cancer among 160 Spanish women with breast cancer and 132 healthy controls. Eight allelic variants of the *NAT2* gene were identified in both cases and controls. The prevalence of the slow-acetylator genotypes was 53% in cases and 51% in controls. All seven patients with lobular breast cancer had the fast-acetylator genotype (47).

Ambrosone et al. (43) examined *NAT2* genetic polymorphisms in relation to cigarette smoking and breast cancer susceptibility in women in western New York state. DNA analyses were performed for three polymorphisms that account for up to 95% of the slow-acetylator phenotype among Caucasians. Among premenopausal women (119 incident cases and 114 population controls), there were no clear patterns of an increased risk of breast cancer associated with smoking by *NAT2* status (43). However, in postmenopausal women (185 cases and 213 controls), *NAT2* modified the association between smoking and the risk of breast cancer. The risk of breast cancer was increased among cigarette smokers with the slow-acetylator genotype but not among those with the rapid-acetylator genotype (43). These findings may explain why cigarette smoking has not been consistently found to be associated with postmenopausal breast cancer in most epidemiologic studies. In addition to the possibility of true biologic effects, the inconsistent findings among premenopausal and postmenopausal women with and without breast cancer may be accounted for by chance variation, since the numbers of premenopausal subjects were smaller.

Millikan et al. (48) examined the association between cigarette smoking and N-acetylation in 498 women with breast cancer and 473 population controls in North Carolina. The study population had approximately equal numbers of African American and white women. Neither *NAT1* nor *NAT2* was associated with a risk of breast cancer. Except among postmenopausal women (n= 252 cases and 255 controls), little evidence of an interactive effect was found between smoking and the *NAT1* or *NAT2* genotype. Among postmenopausal women, the odds ratios for smoking within the past three years was greater among those with the *NAT1*10* genotype (OR = 9.0, 95% CI = 1.9-41.8) than among those with the *NAT1-non*10* genotype (OR = 2.5, 95% CI = 0.9-7.2)(46). The *NAT1*10* genotype, a newly discovered polymorphism of the *NAT1* gene, has been associated with elevated N-acetyltransferase activity (49). The observed odds ratios for smoking within the past three years were also greater among postmenopausal women with the *NAT2* rapid-acetylator genotype (OR = 7.4, 95% CI = 1.6-32.6) than among those with the *NAT2* slow-acetylator genotype (OR = 2.8, 95% CI = 0.4-8.0)(48). Hunter et al. (49) observed little evidence of an association between *NAT2* genotype and risk of breast cancer in a nested case-control study of 466 women with incident breast cancer and 466 matched controls within the Nurses' Health Study.

GLUTATHIONE S-TRANSFERASE

The glutathione S-transferase-M1 (*GSTM1*) gene is located on chromosome 1, and the gene for glutathione S-transferase-T1 (*GSTT1*) is located on chromosome 11q (17, 50). A glutathione S-transferase-P1 (*GSTP1*) gene has also been identified (51).

Glutathione S-transferases detoxify a variety of carcinogens and cytotoxic drugs (for

example, benzo[a]pyrene, monohalomethanes such as methyl chloride, ethylene oxide,

pesticides, and solvents used in industry) by catalyzing the conjugation of a glutathione

moiety to the substrate (50). The incorporation of glutathione increases the molecule's

water solubility and excretability (17). Individuals who are homozygous carriers of

deletions in the *GSTM1* or *GSTT1* gene may have a higher risk of cancer of the breast

and other sites because of their impaired ability to metabolize and eliminate carcinogens

(50). *GSTM1* is polymorphically expressed, and three alleles at the *GSTM1* locus have

been identified: *GSTM1-0* (homozygous deletion genotype), *GSTM1a*, and *GSTM1b* (50).

The null allele (*GSTM1-0*) is present in about 38%-67% of Caucasians and 22%-35% of

African Americans (17). *GSTM1* is not expressed in breast tissue at high levels (50). Two

functionally different genotypes at the *GSTT1* locus have been described: *GSTT1-0*

(homozygous deletion genotype) and *GSTT1-1* (genotypes with one or two undeleted

alleles) (50). A polymorphism of the *GSTP1* gene, *A313G* (changing codon 105 from Ile to

Val), has been identified.

Ambrosone et al. (19) studied 216 postmenopausal Caucasian women with

incident breast cancer and 282 community controls in western New York state. The

authors found no association between breast cancer and the *GSTM1* genotype (OR = 1.1,

95% CI = 0.7-1.6). The results suggested that the null genotype might be associated with

a risk of breast cancer among the youngest postmenopausal women (OR = 2.4, 95% CI =

0.9-6.6). Cigarette smoking did not modify the association between *GSTM1* and the risk

of breast cancer (19). Zhong et al. (52) found no significant differences in the frequency of the *GSTM1* null allele among 197 patients with breast cancer and 225 volunteer controls in Great Britain.

In a nested case-control study within the Nurses' Health Study, no association was found between the *GSTM1* deletion and the development of incident breast cancer (53). The gene deletion polymorphism appeared to confer improved survival, however, and the null genotype was slightly more common among women with prevalent breast cancer (58%) than among controls (51%; age-adjusted OR = 1.3, 95% CI = 0.9-1.9).

Helzlsouer et al. (51) recently assessed the association between glutathione S-transferase polymorphisms and incident breast cancer in a nested case-control study in Washington County, Maryland. Stored blood specimens obtained from volunteers were used in the study. Over a six-year follow-up period, an increased risk of breast cancer was observed among women with the *GSTM1* homozygous deletion (or null) genotype (OR = 2.5, 95% CI = 1.34-4.65). The increase was due primarily to an association with postmenopausal breast cancer (51). A trend toward increased risk was also observed for valine/valine homozygosity in *GSTP1* and for the homozygous deletion (or null) genotype in *GSTT1*. The odds ratio for the combined *GSTM1* null, *GSTT1* null, and one or two copies of *GSTP1* valine (i.e., the higher-risk genotypes) was 3.77 (95% CI = 1.10-12.88)(51). These inconsistent results across studies may be explained by the limited number of subjects available for some analyses or by population differences in other risk factors for breast cancer.

Glutathione S-transferase genotypes have also been examined in relation to the age at which breast cancer was diagnosed in women with a positive family history of breast cancer. Rebbeck et al. (54) studied 185 cases of breast cancer ascertained through hereditary breast cancer clinics in the United States, and found no association between *GSTM1* genotypes and risk of breast cancer. However, the *GSTT1-1* allele was associated with a younger age at first diagnosis than the *GSTT1-0* allele (54). About 40% of the subjects were diagnosed before age 40.

ESTROGEN RECEPTOR POLYMORPHISMS

The biological role of estrogens, including the growth and differentiation of normal mammary tissue, is mediated through high-affinity binding to the estrogen receptor (ER)(55). The ER is a nuclear receptor protein that has an estrogen-binding domain and a DNA-binding domain (56, 57). The presence of ER protein in breast cancer is associated with responsiveness to adjuvant hormone treatment and a more favorable prognosis (55, 56, 58). About two-thirds of breast cancer tumors are ER positive (55). Several epidemiologic studies have examined risk factors for breast cancer according to ER and/or progesterone receptor (PR) status and have produced inconsistent results (56, 59-71). It is still unclear whether breast cancers of different hormone receptor statuses represent etiologically distinct forms of the disease that have different patterns of risk factors (59).

Genetic polymorphisms of the gene that codes for the ER protein have been the subject of increasing interest (70-74). In humans, the ER gene is located on chromosome

6q. The molecular mechanisms that determine ER negativity are poorly understood, and mutations in the coding region have been described in only a small percentage of breast cancer cases (71). More common are genetic polymorphisms of the ER gene that do not alter the encoded amino acid.

Andersen et al. (72) compared the allele frequencies of three RFLP at the ER locus in 360 patients with breast cancer from one hospital in Norway and in 672 convenience controls. The authors analyzed leukocyte DNA from 238 breast cancer patients, tumor DNA from 122 patients, and leukocyte DNA from the controls. The frequency of the allele with the *Xba*I restriction site (in exon 2 or flanking introns of the ER gene) was 1.4 times as great among both groups of breast cancer patients as in the controls (95% CI = 1.0-1.9)(68). The allele frequencies of the *Bst*UI polymorphism (in exon 2 of the ER gene) and the *Pvu*II polymorphism (in intron 1) did not differ between cases and controls. Among the breast cancer patients, there was a borderline association between the *Xba*I restriction site and older age at onset (72).

A more recent study by Andersen et al. (73) in Norway analyzed leukocyte DNA from 143 patients with familial breast and/or ovarian cancer and tumor DNA from 96 patients with breast carcinomas for mutations in the ER gene. Three (2.1%) of the 143 patients (two patients with breast cancer and one with ovarian cancer) with a family history of cancer and 8 (1.1%) of 729 controls had a Gly-to-Cys germ line substitution in codon 160 of the ER gene, which most likely represented a polymorphism (73). The gene frequencies among patients with familial cancer (0.01) and controls (0.005) did not differ significantly (p = .20)(73). Somatic mutations of the ER gene were not detected in any of

the tumors studied. In further study of 229 Swedish patients with familial breast or breast and ovarian cancer, Zelada-Hedman et al. (74) failed to find the Gly-to-Cys germ line substitution in codon 160 of the ER gene.

Roodi et al. (55) examined associations with six ER polymorphisms in a clinic-based series of 188 patients with breast cancer. The authors examined the entire coding region of the ER gene in ER-positive and ER-negative primary breast tumors. Both ER-negative and ER-positive tumors contained neutral polymorphisms in codons 10, 87, 243, 325, and 594 (55). No associations were observed between any of the polymorphic alleles and ER phenotype or tumor type, grade, or stage. However, a statistically significant association was found between the polymorphism in codon 325 and a reported family history of breast cancer (OR = 4.3, 95% CI = 1.8-10.1)(55). The allele frequency was 28% in 34 breast cancer patients with a family history of the disease and 11% in 154 patients without such a family history (p < .001). Somatic mutations of the ER gene that alter the encoded amino acid (missense mutations in codons 69 and 396) were found in only 2 (1%) of 188 breast cancer patients (55). These results indicate that in most primary breast cancers, the ER-negative phenotype is due to deficient ER expression at the transcriptional or posttranscriptional level rather than to mutations in the coding region of the ER gene.

Southey et al. (71) looked for an association between the risk of breast cancer and ER gene polymorphism in codon 325. This was a population-based case-control study of breast cancer in younger women (aged less than forty years) that was carried out in Melbourne and Sydney, Australia. A total of 388 cases and 294 controls were included in

the study. DNA testing of peripheral blood was carried out by using polymerase chain

reaction (PCR) analysis with allele-specific oligonucleotide hybridization. The authors

found no association between ER gene polymorphism in codon 325 and the risk of breast

cancer. They also found no difference in allele frequencies between women with breast

cancer (23%) and controls (21%; p = .4)(71). These inconsistent findings across studies

may be due to the use of a clinic-based series of patients with breast cancer in the study

by Roodi et al. (55) or to the younger ages of the women in the study by Southey et al. (71).

ESTROGEN METABOLITES AND CATECHOL-O-METHYLTRANSFERASE

Increasing evidence indicates that estrogen metabolites, including 16 α-

hydroxyestrone and 2- and 4-hydroxyestrogen catechols, have a role in carcinogenesis

(75-80). Research in animal models has shown that these metabolites have nongenotoxic

cell proliferative effects as well as direct and indirect genotoxic effects (81). For example,

in a study of estradiol-induced carcinogenesis in male Syrian hamsters, catechol

metabolites were shown to be reactive intermediates in carcinogenesis (82). Estrogen

catechols directly and indirectly cause oxidative DNA damage, lipid peroxidation, and,

through their quinone metabolites, DNA adducts (83). In humans, indirectly measured

excretion of estrogen catechols has been shown to be related to breast cancer. Estrogen

catechol excretion rises with breast cancer risk, supporting the hypothesis that increased

oxidation of estradiol to catechols is involved in breast tissue carcinogenesis (84). Some

studies suggest that the 4-hydroxyestrogen catechols are important contributors to

carcinogenesis. In the Syrian hamster model, estrogen treatment resulted in greater 4-

hydroxylation than 2-hydroxylation of estradiol (85). In humans, mammary adenocarcinoma and fibroadenoma microsomes predominantly catalyze 4-hydroxylation, but normal tissue microsomes express comparable 2- and 4-hydroxylase activities (86).

Catechol estrogens are inactivated by methylation of the phenolic hydroxyl group, producing monomethylethers (methoxyestrogens)(87). Methylation is catalyzed by catechol-O-methyltransferase (COMT), which also converts catecholamines to metanephrines. Quercetin, an inhibitor of COMT that by itself does not cause tumor formation in the Syrian hamster model, produced greater tumor size and metastases when coadministered with estradiol than did hormone administration alone (88). Coadministration of quercetin and estradiol also resulted in the accumulation of 2- and 4-hydroxyestrogen catechols (89).

COMT activity is inherited as an autosomal recessive trait (90). Twenty-five percent of Caucasians are homozygous for the low-activity allele ($COMT^{LL}$). The low-activity enzyme is heat labile and has 4- to 5-fold less activity than the product of the high-activity allele ($COMT^{HH}$). The allele sequences differ by one base pair, a G-to-A transition in exon 4, resulting in a valine-to-methionine substitution and the generation of a new *Nia*III site (87). The presence of a G to A transition in exon 4 can be determined with a PCR-based RFLP assay that amplifies a 237 base pair section of exon 4.

Lavigne et al. (83) investigated whether $COMT^{LL}$ was associated with the risk of breast cancer in a study of 115 incident cases of breast cancer and 115 controls matched on age, race, time of blood collection, and menopausal status. In comparisons with

women who had $COMT^{HH}$, neither $COMT^{HL}$ (OR = 1.3, 95% CI = 0.7-2.7) nor $COMT^{LL}$ (OR = 1.4, 95% CI = 0.7-2.9) was associated with breast cancer (83). The corresponding odds ratios were somewhat higher among postmenopausal women (for $COMT^{HL}$ OR = 1.7, 95% CI = 0.8-3.8, and for $COMT^{LL}$, OR = 2.2, 95% CI = 0.9-5.1, but again there were no significant associations. Among overweight women (all women with a body mass index [kg/m^2] greater than 24.47), $COMT^{LL}$ (OR = 3.6, 95% CI = 1.1-12.0) was associated with breast cancer, but $COMT^{HL}$ (OR = 1.8, 95% CI = 0.6-5.6) was not (83).

Thompson et al. (91) examined the association between $COMT$ genetic polymorphisms and the risk of breast cancer in a population-based, age-matched, case-control study of incident breast cancer in western New York state. The low-activity allele was significantly associated with an increased risk of breast cancer among premenopausal women (n=141 cases and 134 controls, OR = 2.1, 95% CI = 1.4-4.3) (91), and the low-activity allele was inversely associated with breast cancer risk among postmenopausal women (n=140 cases and 155 controls, OR = 0.4, 95% CI = 0.2-0.7) (91). These results suggest that the contributions of $COMT$ to the risk of breast cancer may depend on menopausal status (91), but negative results have also been reported (92).

Millikan et al. (92) examined the association of $COMT$ genotype and breast cancer risk in a population-based, case-control study of invasive breast cancer in North Carolina. A total of 654 cases and 642 controls were included, with both African American women and white women represented. The low-activity allele was not significantly associated with an increased risk of breast cancer (multivariate adjusted OR = 0.8, 95% CI = 0.6-1.1) and

odds ratios for *COMT* did not differ among African American and white women. Neither menopausal status nor body mass index strongly modified the association with *COMT* (92).

SUMMARY AND CONCLUSIONS

The etiology of breast cancer cannot be explained by allelic variability at a single locus (8, 50). Instead, the major burden of breast cancer in the population probably results from complex interactions between many genetic and environmental factors over time. Cumulative lifetime exposure to estrogen, estrogen metabolites, and other physiologic factors, as well as environmental exposures, could play an important role in the etiology of breast cancer in genetically predisposed women (8). An improved understanding of the interplay of xenobiotic exposures, endogenous physiology, and genetic variability at multiple loci may help to identify women who are at increased risk for breast cancer (50).

The causality of associations between genetic polymorphisms and breast cancer is uncertain because of the inconsistency across studies, but the associations are biologically plausible. The biological plausibility and biological coherence of associations is less clear for polymorphisms that do not alter biological activity or function and results from animal studies should also be taken into account. The temporal relation between these inherited factors and the onset of breast cancer is clear. The strength of the associations is an important causal criterion that may be more difficult to meet with a multifactorial disease such as breast cancer. The specificity of the associations is a causal criterion unlikely to be met since genetic polymorphisms may influence risk of a

variety of diseases.

Further population-based studies of incident cases of breast cancer, with adequate sample sizes and in racially diverse populations, are needed to look at associations with several genetic polymorphisms and risk factors for breast cancer (exogenous estrogens, reproductive factors, diet, alcohol intake, cigarette smoking, and other environmental exposures), while taking possible causal pathways into account. In studies reported to date, the numbers of cases and controls have often been small with overlapping confidence intervals. Of particular interest are further studies of genes that code for those cytochrome P450 enzymes that are involved in the metabolism and transport of estrogen (for example, *CYP17*), as well as studies of gene-environment interactions and gene-gene-environment interactions (for example, *NAT2* and *CYP1A1* polymorphisms and cigarette smoking in relation to breast cancer risk)(20, 28, 37, 43, 49). The molecular epidemiology studies of breast cancer that have been carried out to date have rarely looked at a variety of potential gene-environment interactions or explored associations and interactions with more than one genetic polymorphism. In addition, few studies have examined multiple endogenous factors and genetic polymorphisms in relation to breast cancer risk in women, while taking causal pathways into account. Studies designed to answer such questions are now under way in Los Angeles, Hawaii, North Carolina, and other localities (37, 93). The National Institute for Environmental Health Sciences has also planned an Environmental Genome Project to look at genetic variants that are associated with environmental susceptibility (94).

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